Remarks/Arguments

Claims 1, 5-9, 13, 14, 16-23, 27, 28, 33, 34, 36-43 and 50-60 are pending in the application. Claims 1, 23, 43, 50 and 55 have been amended to indicate that the maturation medium does not contain auxin or cytokinin. Support for these amendments can be found in the specification at least at page 5, lines 11-13, page 8, line23-page 9, line 1, page 9, lines 15-19 and page 12, lines 3-5. Claims 1, 23, 43, 50 and 55 have also been amended to indicate that the embryos are immature during induction, maintenance and prematuration. Support for the amendment to these claims can be found in the specification at least at page 5, lines 5-6 and 20.

Interview Summary

Applicants thank the Examiner for the courtesies extended in the telephonic interview with Applicants' undersigned representative on April 22, 2008. In the interview, the Attree and Fan references and the application of these references to the pending claims were discussed. In addition the objections to the claims containing the term "less than" were discussed. The Examiner explained that she considered these claims to include 0% lactose and thus be improper dependent claims. The Examiner and Applicants' representative also noted that claim 12 was mistakenly included in the §103 rejection over Attree and Handley and that claim 14 was omitted from this rejection. The proper claims are addressed in the response.

Claim Objections

Claims 5, 20, 27, 41, 54 and 60 were objected to as being in improper dependent form for failing to further limit the subject matter of a previous claim. The Examiner asserts that these claims are broader than claim 1 because claim 1 requires the presence of lactose in the nutrient medium and claims 5, 20, 27, 41, 54 and 60 each recite that lactose is less than a certain % of the medium. Specifically, the Examiner contends that these claims encompass nutrient medium comprising 0% lactose. Applicants respectfully assert that according to the MPEP claims in dependent form shall be construed to include all the limitations of the claim incorporated by reference into the dependent claim. MPEP § 608.01(n). Thus, each of claims 5, 20, 27, 41, 54 and 60 require that lactose is present in some measurable amount up to the percentage claimed. Because the claims cannot be construed to exclude the limitations of the independent claim from which they depend, these claims are in proper dependent form. Applicants respectfully request that the objection be withdrawn and the claims allowed.

Rejections Under 35 U.S.C. § 103(a)

Claims 1, 5-9, 13-14, 16-23, 27, 28, 33-34, and 36-43 were rejected as unpatentable over Attree (U.S. Patent No. 6,627,441) in view of Handley (U.S. Patent No. 5,491,090). The Examiner contends that Attree teaches a method of reproducing mature somatic embryos in all conifers, which includes culturing in media containing 3% sucrose, 30μM ABA, 10% PEG and 3.32% lactose (Table 5 and column 26, lines 35-38). The Examiner contends that this medium is used during the prematuration stage because it is used after proliferation and before maturation and because it involves reduction of auxin and cytokinin and a change in the water stress with

the addition of ABA. The Examiner then alleges that Handley teaches a method of regenerating *Pinus taeda* wherein the initiation and maintenance media contain a sugar selected from glucose, maltose, sucrose, melezitose and a combination thereof. The Examiner then contends it would have been obvious to one skilled in the art to reproduce coniferous somatic embryos with a nutrient medium containing lactose and an additional sugar as taught by Attree and to modify the prematuration medium as taught by Handley.

The passage and Table in Attree relied upon by the Examiner refers to a maturation medium and not an induction, maintenance or prematuration medium as recited in independent claims 1, 23 and 43 of the instant application. At column 26, line 26-32, Attree indicates that the embryos were precultured (prematuration) in $1/20^{th}$ strength hormone medium (containing reduced amounts of auxin and cytokinin) for one week prior to transfer to maturation medium and that once in maturation medium the media was changed weekly to the media indicated in Table 5. The media in Table 5 of Attree do not contain any auxin or cytokinin. Additionally, these media allow maturation of the embryos to occur, as shown in Figure 2 of Attree. Mature embryos will not develop in prematuration media. Thus, the media the Examiner points to are maturation media and not prematuration media.

Attree does teach that lactose can be used in the maturation step of conifer somatic embryogenesis and the Examiner notes that because of this one of skill in the art may have tried to use lactose in the induction, maintenance or prematuration media. The Examiner is impermissibly using hindsight to piece together Applicants' invention. Attree makes clear that the lactose in the maturation media was used as an osmoticum to increase the water stress on the embryos and encourage maturation. See column 26, lines 34-35 ("water potential was increased

by adding lactose"). Thus, Attree was using lactose, not as a carbon source, but instead as a means of increasing the water stress on the cells. As detailed in the appended declarations of Attree and Fowke under 37 C.F.R. §1.132, increased water stress during maturation enhances development into mature embryos capable of germination. See Attree Declaration at 8-10 and Fowke Declaration at 6-9. These effects, while being important for maturation of the embryo, are the opposite of the desired effects during induction, maintenance and prematuration of the embryos. In induction, maintenance and prematuration, a low water stress (osmoticum) is desired. Thus, the disclosure of Attree that lactose could be used as an osmoticum in the maturation medium would actually discourage the use of lactose in the induction, maintenance or prematuration stages of somatic embryogenesis by others of skill in the art. Thus, Attree actually teaches away from the present invention.

Prior to the present application, lactose was not believed to be metabolized by conifer cells and non-metabolizable sugars would not normally be added to the induction, maintenance or prematuration media. The results presented in Example 5 of the pending application demonstrate that lactose and galactose are utilized by conifer cells. The fact that lactose could be used as a carbon source was unexpected as noted in the specification at least at page 6, lines 6-9, in Example 5, page 13-14 and Example 5.1, page 14. Without the knowledge that lactose could be used as a carbon source, there would be no reason to add lactose to the induction, maintenance or prematuration media. See Attree Declaration at 11 and Fowke Declaration at 10. Notably, Handley mentions several other sugars as useful in these stages of culture, but does not mention galactose-containing sugars or lactose. The absence of these sugars from Handley

further supports the position that it was not obvious to use lactose and an additional sugar during any of induction, maintenance or prematuration. Handley also does not teach an induction, maintenance or prematuration medium containing lactose and an additional sugar as recited in the independent claims.

Not only were the results demonstrating that lactose and galactose could be metabolized by conifer cells unexpected, no one could have predicted that use of lactose and an additional sugar in the induction, maintenance and/or prematuration media would have produced such a dramatic increase in the number of somatic embryos produced per gram of fresh weight tissue as compared to other sugars. See Attree Declaration at 12-13 and Fowke Declaration at 11. These results were demonstrated throughout the Examples. This represents a significant improvement in the field because maintenance and bulk-up of tissues is a large expense and by generating higher numbers of embryos per gram of tissue the costs of somatic embryogenesis can be decreased significantly. The unexpected benefits of using a galactose-containing sugar as compared to other more traditionally used sugars were noted in the specification at least at page 6, lines 23-25 and page 8, lines 15-21 and are noted in the Declarations of Attree and Fowke. These unexpected benefits seem to be generic to conifers as all three conifers tested demonstrated a significant improvement in the number of somatic embryos produced per gram of tissue when a galactose-containing sugar was used in induction, maintenance and/or prematuration media.

Therefore, the combination of Attree and Handley do not teach or suggest "a nutrient medium selected from the group consisting of induction medium, maintenance medium and prematuration medium, wherein the nutrient medium comprises lactose and an additional sugar"

as recited in claims 1, 23 and 43. Claims 5-9, 13-14, 16-22, 27, 28, 33-34 and 36-42 all depend from claims 1 or 23 and are not obvious over the combination of Attree and Handley for at least the same reasons as stated for claims 1, 23 and 43. Applicants respectfully request that the rejection be withdrawn.

Claims 50-54 were rejected under 35 U.S.C. § 103(a) as unpatentable over Fan (U.S. Patent No. 6,689,609) in view of Handley. The Examiner contends that the claims are "drawn to a method of reproducing somatic embryos of *Pinus taeda* or hybrid thereof comprising growing an explant in induction, maintenance or prematuration medium, comprising between 1.0% and 6.0% lactose for the development of the explant to cotyledon stage suitable for germination." See Office action at page 7. Applicants respectfully assert that claim 50 recites that "the prematuration medium is used to prepare the embryogenic culture for transfer to maturation medium and subsequent development of cotyledonary stage embryos suitable for germination." The prematuration medium does not allow formation of mature embryos suitable for germination, the culture must be transferred to a maturation medium.

The Examiner alleges that the phase two growth of somatic embryos in Fan requires a carbohydrate source, such as lactose in the range of 3-6% and that phase two in Fan is equivalent to the maintenance step in the current application. The Examiner acknowledges that Fan does not teach *Pinus taeda* but alleges that one of skill in the art would have been motivated to combine the teachings of Fan with those of Handley because *Pinus taeda* is an important timber crop. In addition, the Examiner alleges that one of skill in the art would have had a reasonable expectation of success in the combination because the method of Fan is used with other species

of pines. The Examiner notes that claims do not state the age of the starting material. Applicants have amended claim 50 to clearly indicate that the claim relates to immature somatic embryos, not the mature somatic embryos taught as the starting material in Fan. See Fan column 10, lines 43-44. Germination, which is taught in Fan, is the final step in somatic embryogenesis and is distinct from induction, maintenance and prematuration in that the starting material for germination is a mature somatic embryo that has completed the maturation process, not an immature somatic embryo.

Therefore, Fan does not teach or suggest using medium comprising lactose in the induction, maintenance or prematuration steps of the somatic embryogenesis process as recited in independent claim 50. As stated above, Handley does not cure this deficiency. Claims 51-54 all depend from claim 50 and are not obvious over the combination of Fan and Handley for at least the same reasons as stated for claim 50. Applicants respectfully request that the rejection be withdrawn.

Claims 55-60 were rejected under 35 U.S.C. § 103(a) as unpatentable over Coke (U.S. Patent No. 5,534,433) in view of Pullman (U.S. Patent No. 6,492,174). The Examiner alleges that Coke teaches a method for embryo development (which the Examiner characterizes as maintenance) of *Pinus taeda*, using a combination of sucrose and maltose in the medium. The Examiner contends that the embryo development medium of Coke is equivalent to the maintenance medium of the current claims. The Examiner states the media are equivalent because the maintenance medium is used to grow and maintain the embryogenic culture in the claims and the embryo development medium of Coke is used to grow and develop the

embryogenic culture. See Office action at page 10-11. The Examiner has stated the clear distinction between these media. The maintenance media of the current claims is designed to allow the immature embryogenic culture to proliferate (grow and maintain). The embryo development medium of Coke is for the purpose of producing mature cotyledonary stage embryos (grow and develop means develop cotyledons). See Coke at column 7, lines 32-40. Somatic embryos will not mature into cotyledonary stage embryos on induction, maintenance or prematuration media. As discussed above in detail in regards to the rejection over Attree and Handley, use of a combination of sugars in a maturation medium would not provide one of skill in the art with any reason to add a galactose-containing sugar and an additional sugar to the maintenance or prematuration media.

The Examiner alleges that Pullman teaches initiation of *Pseudotsuga menziesii* and *Pinus radiata* embryogenic cultures in media containing 1-1.5% maltose, glucose, fructose, sucrose, galactose or a combination thereof. The Examiner then alleges that it would have been obvious to one of skill in the art to reproduce coniferous somatic embryos in medium containing two sugars as taught by Coke and to modify the sugars by using galactose as the primary sugar as taught by Pullman. Pullman does not cure the deficiencies of Coke because Pullman does not teach or suggest media for use in maintenance or prematuration and is limited to media for improving initiation (induction). Neither of these references relates to the steps in somatic embryogenesis claimed and the references in combination do not teach or fairly suggest use of a combination of a galactose-containing sugar with an additional sugar at these stages of embryogenesis.

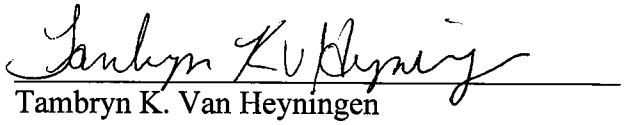
Therefore, one of skill in the art with the teachings of Coke and Pullman would not have expected a combination of a galactose-containing sugar and an additional sugar in the maintenance or prematuration steps of somatic embryogenesis to be useful. In addition, as discussed above in relation to the rejection over Attree and Handley, the use of a galactose-containing sugar and an additional sugar in the maintenance and prematuration stages yielded unexpected results which could not have been predicted from the teachings of Coke and Pullman. Claims 56-60 all depend from claim 55 and are not obvious over the combination of Coke and Pullman for at least the same reasons as stated for claim 55. Applicants respectfully request that the rejection be withdrawn.

Conclusion

Accordingly, Applicants respectfully request withdrawal of the rejections and allowance of the claims.

Respectfully submitted,

Date: May 16, 2008



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